

We Claim:

1. A method to modulate the expression of a target gene in plant cells, which method comprises providing plant cells with a zinc finger protein, said zinc finger protein being capable of specifically binding to a target nucleotide sequence, or a complementary strand thereof, within a target gene, and allowing said zinc finger protein binding to said target nucleotide sequence, whereby the expression of said target gene in said plant cells is modulated.

2. The method of claim 1, wherein the zinc finger protein is exogenously added to the plant cells and the plant cells are maintained under conditions such that the zinc finger protein binds to the target nucleotide sequence and regulates the expression of the target gene in the plant cells.

3. The method of claim 1, wherein a nucleotide sequence encoding the zinc finger protein is expressed in the plant cells and the plant cells are maintained under conditions such that the expressed zinc finger protein binds to the target nucleotide sequence and regulates the expression of the target gene in the plant cells.

4. A method to modulate the expression of a target gene in plant cells, which method comprises:

a) providing plant cells with an expression system for a zinc finger protein, said zinc finger protein being capable of specifically binding to a target nucleotide sequence, or a complementary strand thereof, within a target gene; and

b) culturing said plant cells under conditions wherein said zinc finger protein is produced and binds to said target nucleotide sequence, whereby expression of said target gene in said plant cells is modulated.

5. The method of claim 1, wherein the target nucleotide sequence is endogenous or exogenous to the target gene.

6. The method of claim 1, wherein the target nucleotide sequence is upstream of, downstream of, or within the coding region of the target gene.

7. The method of claim 1, wherein the target nucleotide sequence is DNA, RNA, PNA or a combination thereof.

8. The method of claim 1, wherein the target nucleotide sequence is a promoter of a regulatory protein.

9. The method of claim 1, wherein the target nucleotide sequence comprises 3, 6, 9, 12, 15 or 18 nucleotides.

10. The method of claim 1, wherein the target nucleotide sequence comprises 18 nucleotides and wherein the zinc finger protein is a hexadactyl zinc finger protein.

11. The method of claim 1, wherein the targeted nucleotide sequence is of the formula $(GNN)_n$, and wherein N is any one of the A, T, C or G and n is an integer from 1 to 6.

12. The method of claim 11, wherein the n is 6.

13. The method of claim 1, wherein the target nucleotide sequence is endogenous to the plant but is in a non-naturally-occurring location.

14. The method of claim 1, wherein the plant cells comprise at least two copies of the same or different target nucleotide sequence(s).

15. The method of claim 14, wherein each target nucleotide sequence is located within a different target gene, whereby more than one different target genes are modulated.

16. The method of claim 1, wherein the target gene encodes a product that affects biosynthesis, modification, cellular trafficking, metabolism and degradation of a peptide, a protein, an oligonucleotide, a nucleic acid, a vitamin, an oligosaccharide, a carbohydrate, a lipid, or a small molecule.

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17. The method of claim 16, wherein the target gene encodes a protein or an RNA.

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18. The method of claim 1, wherein the target gene encodes myoinositol 1-phosphate synthase.

19. The method of claim 1, wherein the target gene encodes a protein and the expression of said encoded protein is modulated.

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20. The method of claim 19, wherein the protein whose expression being modulated is heterologous to the plant cell.

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21. The method of claim 20, wherein the protein whose expression being modulated is an antibody.

22. The method of claim 19, wherein the expression of the protein is activated.

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23. The method of claim 19, wherein the protein whose expression being modulated participates in a metabolic pathway or controls a metabolic pathway.

24. The method of claim 23, wherein the metabolic pathway is an anabolic or a catabolic pathway.

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25. The method of claim 23, wherein the metabolic pathway is for a molecule selected from the group consisting of a vitamin, a taste molecule, an anti-oxidant, a sugar and a flavanoid molecule.

26. The method of claim 25, wherein the taste molecule is a bad taste molecule.

5 27. The method of claim 23, wherein the metabolic pathway is heterologous to the plant cell.

28. The method of claim 23, wherein the metabolic pathway enhances an input or output trait in a plant or seed.

10 29. The method of claim 19, wherein the target gene encodes an enzyme, a transport protein, a nutrient or storage protein, a contractile or motile protein, a structural protein, a defense protein or a regulatory protein.

15 30. The method of claim 19, wherein the target gene encodes an enzyme or a co-factor in a metabolic pathway.

20 31. The method of claim 1, which method is used for treating a disorder in the plant cells, wherein the disorder is associated with abnormal expression of the target gene.

32. The method of claim 1, wherein the zinc finger protein is linked to a protein which activates or represses gene expression.

25 33. The method of claim 32, wherein the zinc finger protein is linked to the protein which activates or represses gene expression as a fusion protein.

30 34. The method of claim 32, wherein the protein which activates gene expression comprises an activator domain of a regulatory protein.

35. The method of claim 32, wherein the protein which activates or represses gene expression comprises an active domain of a nucleic acid modifying protein.

5 36. The method of claim 1, wherein the zinc finger protein binds to the complementary strand of the target nucleotide sequence.

10 37. The method of claim 1, wherein the zinc finger protein specifically binds to an effector domain of the target sequence and whereby the expression of the target gene is modulated by competitive inhibition of said effector domain.

38. The method of claim 37, wherein the zinc finger protein does not comprise an effector domain.

15 39. The method of claim 1, wherein the zinc finger protein comprises an effector domain active in the host plant cells.

40. The method of claim 1, wherein the zinc finger protein comprises a plurality of finger regions.

20 41. The method of claim 40, which comprises linker regions among the plurality of finger regions.

25 42. The method of claim 1, wherein the zinc finger protein comprises at least two 3-finger region and the linker region between any said two 3-finger region is from about 2 to about 10 amino acid residues in length.

43. The method of claim 42, wherein the linker region between any said two 3-finger region is about 5 amino acid residues in length.

30 44. The method of claim 1, wherein the zinc finger protein comprises a framework from a plant zinc finger protein.

45. The method of claim 1, wherein the zinc finger protein is a synthetic zinc finger protein, or a non-naturally-occurring zinc finger protein in the host plant.

5 46. The method of claim 1, wherein the zinc finger protein is selected from the group consisting of ZFPm1, ZFPm2, ZFPm3, ZFPm4 and ZFPAp3.

10 47. The method of claim 1, wherein the zinc finger protein is not a zinc finger-nucleotide binding polypeptide variant comprising at least three zinc finger modules that bind to a target cellular nucleotide sequence and modulate the transcriptional function of the cellular nucleotide sequence, wherein the amino acid sequence of each zinc finger module that binds a target cellular nucleotide comprises two cysteines and two histidines whereby both cysteines are amino proximal to both histidines and wherein each of three modules of said variant has at least one amino acid sequence modification.

15 48. The method of claim 1, wherein the plant cells are monocot or dicot plant cells.

20 49. The method of claim 1, wherein the plant cells are included within an intact plant or constitute all the cells of an intact plant.

50. The method of claim 1, wherein the plant cells are protoplasts or spheroplasts.

25 51. The method of claim 1, wherein the modulation of the gene expression is activation or repression.

30 52. The method of claim 1, wherein the modulation of the gene expression is at least two fold.

53. The method of claim 52, wherein the modulation is at least five fold repression.

54. The method of claim 52, wherein the modulation is at least two fold activation.

55. The method of claim 1, wherein the modulation changes the phenotype of the plant cells, the tissue of the plant or the whole plant.

56. The method of claim 4, wherein the plant cells are contained in an *in vitro* culture.

57. The method of claim 4, wherein the culturing is in planta.

58. The method of claim 4 wherein the expression system comprises an inducible promoter.

59. The method of claim 4, wherein the expression of the zinc finger protein is controlled by a tissue-specific promoter and whereby tissue-specific modulation of the target gene expression is obtained.

60. The method of claim 59, wherein the tissue is selected from the group consisting of calli, meristem, leave, root and organ explant in tissue culture.

61. The method of claim 4, wherein the zinc finger protein is expressed in a specific organelle.

62. The method of claim 61, wherein the organelle is selected from the group consisting of a mitochondria, a nucleus, a plastid and a vacuole.

63. The method of claim 62, wherein the plastid is selected from the group consisting of a chloroplast, a leucoplast, an aravloplast and a chromoplast.

5 64. The method of claim 4, wherein the zinc finger protein is stably integrated in a specific organelle.

65. The method of claim 4, wherein the zinc finger protein is targeted to a specific organelle.

10 66. The method of claim 65, wherein the zinc finger protein is targeted to plastid via a plastid transit peptide, to chloroplast via a chloroplast transit peptide, to mitochondrial via a mitochondrial target peptide or to nucleus via a nuclear targeting peptide.

15 67. The method of claim 4, wherein the expression is transient or stable.

68. The method of claim 4, wherein the zinc finger protein comprises preferred codons of the host plant.

20 69. The method of claim 68, wherein the zinc finger protein comprises preferred translational start codon of the host plant.

25 70. A method of modulating a level of a compound in a plant cell, which method comprises expressing in a plant cell a zinc finger protein that specifically binds to a target nucleotide sequence within a target gene to modulate expression of said target gene which is involved in a compound's metabolism in said plant cell, whereby the level of said compound in said plant cell is modulated.

30 71. The method of claim 70, wherein the compound is phytic acid.

72. The method of claim 70, wherein the target gene encodes AP3.

73. The method of claim 70, wherein the zinc finger protein is not a zinc finger-nucleotide binding polypeptide variant comprising at least three zinc finger modules that bind to a target cellular nucleotide sequence and modulate the transcriptional function of the cellular nucleotide sequence, wherein the amino acid sequence of each zinc finger module that binds a target cellular nucleotide comprises two cysteines and two histidines whereby both cysteines are amino proximal to both histidines and wherein each of three modules of said variant has at least one amino acid sequence modification.

74. An expression vector for modulating gene expression in plant cells, which expression vector comprises a nucleotide sequence encoding a zinc finger protein, said zinc finger protein is capable of specifically binding to a target nucleotide sequence, or a complementary strand thereof, within a target gene whose expression is to be modulated by said zinc finger protein in plant cells.

75. The expression vector of claim 74, wherein the zinc finger protein is not a zinc finger-nucleotide binding polypeptide variant comprising at least three zinc finger modules that bind to a target cellular nucleotide sequence and modulate the transcriptional function of the cellular nucleotide sequence, wherein the amino acid sequence of each zinc finger module that binds a target cellular nucleotide comprises two cysteines and two histidines whereby both cysteines are amino proximal to both histidines and wherein each of three modules of said variant has at least one amino acid sequence modification.

76. A genetically modified plant cell, which cell comprises an expression system for a zinc finger protein, said zinc finger protein is capable of specifically binding to a target nucleotide sequence, or a complementary strand thereof, within a target gene whose expression is to be modulated by said zinc finger protein.

77. The genetically modified plant cell of claim 76, wherein the target nucleotide sequence is endogenous or exogenous to the targeted gene.

5 78. The genetically modified plant cell of claim 76, wherein the target gene is endogenous or exogenous to the plant cells.

79. The genetically modified plant cell of claim 76, which is contained in an intact plant.

10 80. The genetically modified plant cell of claim 76, wherein the zinc finger protein controls its own expression by binding to a target sequence within the zinc finger protein gene.

15 81. The genetically modified plant cell of claim 76, wherein the zinc finger protein controls its own expression by binding to a first target sequence within the zinc finger protein gene and controls the expression of the target gene by binding to a second target sequence within the target gene.

20 82. The genetically modified plant cell of claim 81, wherein the first target sequence within the zinc finger protein gene is different from the second target sequence within the target gene.

25 83. The genetically modified plant cell of claim 76, wherein the zinc finger protein gene is further controlled by a second promoter.

84. The genetically modified plant cell of claim 83, wherein the second promoter is inducible.

30 85. The genetically modified plant cell of claim 76, wherein the zinc finger protein comprises at least two zinc finger sequences.

86. The genetically modified plant cell of claim 85, wherein the zinc finger protein comprises from about 2 to about 6 zinc finger sequences.

5 87. The genetically modified plant cell of claim 85, wherein the zinc finger protein comprises from about 3 to about 6 zinc finger sequences.

88. The genetically modified plant cell of claim 76, which is selected from the group consisting of a tobacco, tomato, potato, banana, soybean, pepper, wheat, rye, rice, spinach, carrot, maize and corn cell.

10 89. The genetically modified plant cell of claim 76, wherein the zinc finger protein expressed in the plant cell is not a zinc finger-nucleotide binding polypeptide variant comprising at least three zinc finger modules that bind to a target cellular nucleotide sequence and modulate the transcriptional function of the cellular nucleotide
15 sequence, wherein the amino acid sequence of each zinc finger module that binds a target cellular nucleotide comprises two cysteines and two histidines whereby both cysteines are amino proximal to both histidines and wherein each of three modules of said variant has at least one amino acid sequence modification.

20 90. A genetically modified plant cell transformed with a nucleic acid comprising a functional geminiviral replicase gene operably linked to a fruit ripening-dependent promoter.

25 91. A genetically modified plant cell, which cell comprises an exogenous zinc finger protein that specifically binds to a target nucleotide sequence in said plant cell wherein said exogenous zinc finger protein is constitutively expressed.

30 92. A genetically modified plant cell, which cell comprises an exogenous zinc finger protein that specifically binds to a target nucleotide sequence in said plant cell wherein said exogenous zinc finger protein is inducibly expressed.

93. A genetically modified plant tissue, which tissue comprises the genetically modified plant cell of claim 76.

94. A genetically modified plant seed, which seed comprises the genetically modified plant cell of claim 76.

95. The genetically modified plant seed of claim 94, which is selected from the group consisting of a tobacco, tomato, potato, banana, soybean, pepper, wheat, rye, rice, spinach, carrot, maize and corn seed.

96. A genetically modified plant seed transformed with a nucleic acid comprising a functional geminiviral replicase gene operably linked to a fruit ripening-dependent promoter.

97. A genetically modified plant seed, which seed comprises the genetically modified plant cell of claim 89.

98. A plant that is regenerated from a plant transformed with the expression vector of claim 74.

99. A method to modulate expression in a plant cell, which method comprises culturing the plant cell of claim 76.

100. The method of claim 99, wherein the plant cell is cultured in planta.

101. A zinc finger protein comprising a zinc finger nucleic acid binding domain and an effector domain, wherein said effector domain comprises an active domain of a restriction enzyme, an active domain of a nucleic acid modifying protein, a label or a modification.

102. The zinc finger protein of claim 101, wherein the nucleic acid modifying protein is a nucleic acid methylase.

103. A zinc finger protein that is selected from the group consisting of ZFPm1, ZFPm2, ZFPm3, ZFPm4 and ZFPAp3.

104. A zinc finger protein that is recognizable by an antibody that specifically binds to a zinc finger protein selected from the group consisting of ZFPm1, ZFPm2, ZFPm3, ZFPm4 and ZFPAp3.

105. The zinc finger protein of claim 104, which retains gene expression modulation activity of ZFPm1, ZFPm2, ZFPm3, ZFPm4 or ZFPAp3.

106. An antibody that specifically binds to a zinc finger protein selected from the group consisting of ZFPm1, ZFPm2, ZFPm3, ZFPm4 and ZFPAp3.

107. The antibody of claim 106, which is a monoclonal antibody.

108. An isolated nucleic acid fragment, comprising a sequence of nucleotides encoding a zinc finger protein that is selected from the group consisting of ZFPm1, ZFPm2, ZFPm3, ZFPm4 and ZFPAp3.

109. The isolated nucleic acid fragment of claim 108, which is DNA or RNA.

110. An isolated nucleic acid fragment, which is hybridizable to the nucleic acid fragment of claim 108.

111. The isolated nucleic acid fragment of claim 110, which is hybridizable to the nucleic acid fragment of claim 108 under high stringency condition.

112. A plasmid, comprising the nucleic acid fragment of claim 108.

113. A cell, comprising the plasmid of claim 112.

114. The cell of claim 113, wherein the cell is selected from the group
5 consisting of a bacterial cell, a yeast cell, a fungal cell, a plant cell, an insect cell and an animal cell.

115. A method for producing a zinc finger protein, comprising: growing the
10 cell of claim 113 under conditions whereby the zinc finger protein is expressed by the cell; and recovering the expressed zinc finger protein.

116. An assay method for determining a suitable position in a gene for
regulating gene expression in plant cells, which method comprises:

- 15 a) providing a target gene which contains a nucleotide sequence encoding a reporter protein within the coding region of said target gene and a target nucleotide sequence at a predetermined location within said target gene;
- b) contacting said target gene with a regulatory factor comprising a zinc finger protein specific for said target nucleotide sequence; and
- 20 c) assessing the level of expression of said reporter gene in the presence and absence of said contacting;
- wherein a change in the level of expression of said reporter gene in the presence as opposed to the absence of said contacting identifies said position of said target nucleotide sequence as a position suitable for controlling expression of said target gene in plant cells.

25 117. The assay method of claim 116, wherein the zinc finger protein is not a zinc finger-nucleotide binding polypeptide variant comprising at least three zinc finger modules that bind to a target cellular nucleotide sequence and modulate the transcriptional function of the cellular nucleotide sequence, wherein the amino acid
30 sequence of each zinc finger module that binds a target cellular nucleotide comprises two cysteines and two histidines whereby both cysteines are amino proximal to both

histidines and wherein each of three modules of said variant has at least one amino acid sequence modification.

5 118. A fusion protein, which fusion protein comprises a zinc finger of 2C7 and an effector domain of SID.

119. A zinc finger protein that is recognizable by an antibody that specifically binds to a fusion protein comprising a zinc finger of 2C7 and an effector domain of SID3.

10 120. The zinc finger protein of claim 119, which retains gene expression modulation activity of the fusion protein comprising a zinc finger of 2C7 and an effector domain of SID3.

15 121. The fusion protein of claim 118, which is encoded by the nucleotide sequence set forth in SEQ ID NO:5 or SEQ ID NO:66.

122. An antibody that specifically binds to a fusion protein comprising a zinc finger of 2C7 and an effector domain of SID3.

20 123. The antibody of claim 122, which is a monoclonal antibody.

124. An isolated nucleic acid fragment, comprising a sequence of nucleotides encoding a fusion protein comprising a zinc finger of 2C7 and an effector domain of SID3.

25 125. The isolated nucleic acid fragment of claim 124, which is DNA or RNA.

126. An isolated nucleic acid fragment, which is hybridizable to the nucleic acid fragment of claim 124.

127. The isolated nucleic acid fragment of claim 126, which is hybridizable to the nucleic acid fragment of claim 124 under high stringency condition.

128. The isolated nucleic acid fragment of claim 124, which has the nucleotide sequence set forth in SEQ ID NO:5 or SEQ ID NO:66.

129. A plasmid, comprising the nucleic acid fragment of claim 124.

130. A cell, comprising the plasmid of claim 129.

131. The cell of claim 130, wherein the cell is selected from the group consisting of a bacterial cell, a yeast cell, a fungal cell, a plant cell, an insect cell and an animal cell.

132. A method for producing a fusion protein, comprising: growing the cell of claim 130 under conditions whereby the fusion protein is expressed by the cell; and recovering the expressed fusion protein.

133. The method of claim 1, wherein the zinc finger protein comprises a framework (or backbone) derived from a naturally occurring zinc finger protein.

134. The method of claim 1, wherein the zinc finger protein comprises a framework (or backbone) derived from a zinc finger protein comprising a C2H2 motif.

135. The method of claim 134, wherein the protein or peptide sequence within the β sheet of the C2H2 motif is not changed from its natural sequence.

136. The method of claim 1, wherein the zinc finger protein comprises a framework (or backbone) derived from a zinc finger protein that is naturally functional in plant cells.

137. The method of claim 136, wherein the framework (or backbone) comprises a motif selected from the group consisting of a C3H zinc finger, a QALGGH motif, a RING-H2 zinc finger motif, a 9 amino acid C2H2 motif, a zinc finger motif of Arabidopsis LSD1 and a zinc finger motif of BBF/Dof domain proteins.

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138. The method of claim 1, wherein the zinc finger protein comprises a framework (or backbone) derived from a zinc finger protein that is known in the art as of January 19, 2001.

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